SOME FORMS OF GILL DISEASE IN PENAEID SHRIMP[1]

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ABSTRACT

Gill disease in penaeid shrimp is a complex of several afflictions, any of which may result in death of affected animals either by outright destruction of the gills or by suffocation resulting from mechanical blockage of gas exchange across the surface of the gill lamellae. Organisms demonstrated to cause gill disease in penaeids include an imperfect fungus that belongs to the genus Fusarium, at least two types of epicommensal peritrichs that belong to the genera Zoothamnium and Lagenophrys, and a filamentous bacterium that superficially resembles Leucothrix mucor.

"Black gills" or melanization of the gill process is a sign of several forms of gill disease and is not a disease in itself. "Black gills" occur in shrimp with heavy infestations of Lagenophrys.

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"Black gills" are not usually seen in animals that have heavy infestations on the surface of the gills of either the epicommensal peritrich Zoothamnium or the filamentous bacterium. Detritus and algae are often trapped by these epicommensals, and this results in gills that range in color from green to dark brown.

Demonstrable histological damage to the gills does not occur in shrimp dying from heavy infestations of the filamentous bacterium or Zoothamnium. Histopathological changes are readily demonstrated in animals having Fusarium infections and in animals with the epicommensal Lagenophrys sp.

INTRODUCTION

For aquaculture to be commercially feasible in many areas of the United States, the economic requirement that maximum numbers of individuals utilize minimum quantities of space and water must be realized. The most practical approach to satisfying this requirement is the employment of intensive culture methods. Neal (1973) described intensive culture methods as those in which fish or shellfish are reared in man-made ponds, raceways, or tanks where environmental control is exercised, contrasting these intensive methods to extensive culture where natural bodies of water are utilized with few modifications of the environment. High culture densities are not usually possible when extensive culture methods are used, and disease control and prevention in these systems is seldom possible or practical. Diagnosis, treatment, and prophylaxis of infectious diseases are both possible and practical when intensive culture techniques are used. The crowding that is a necessary part of intensive culture methodology virtually guarantees that both infectious and noninfectious diseases will occur. Early experiences with disease in intensive shrimp culture have shown that diseases of the gills are among the most serious.

Various forms of gill disease in penaeid shrimp have already been described from extensive and intensive culture systems (Lightner, in press). Organisms so far demonstrated to cause gill disease in penaeids include species of imperfect fungi that belong to the genus Fusarium, at least two types of epicommensal peritrichs that belong to the genera Zoothamnium and Lagenophrys, and a filamentous bacterium that superficially resembles Leucothrix mucor. Presented in this paper are some new data on these forms of gill disease, particularly those occurring in intensive culture.

MATERIALS AND METHODS

Most of the shrimp for these studies were obtained from the National Marine Fisheries Service shrimp hatchery and rearing facility in Galveston, Texas, and from the University of Arizona-University of Sonora shrimp farms at Tucson, Arizona, and Puerto Peñasco, Sonora, Mexico. Some wild shrimp from commercial bait dealers on Galveston Bay were also used.

Methods of identification of presumed pathogens are given in the appropriate sections for each of the diseases discussed.

Shrimp selected for histological examination were fixed live in either 10% phosphate-buffered formalin, Carnoy's fixative, or Davidson's fixative. In the case of small shrimp (under 60 mm in total length) the cuticle over the hepatopancreas and over the abdominal musculature was opened with scissors to enhance fixative penetration. In the case of larger shrimp, body regions that contained the organs or tissues of interest (e.g., the gills) were removed and fixed separately. Embedding, sectioning, and staining were accomplished using routine histological methods.

RESULTS AND DISCUSSION

Gill Disease Due to Fusarium sp.

Imperfect fungi of the genus Fusarium have been reported from the Kuruma prawn, Penaeus japonicus, in Japan (Egusa and Ueda, 1972), from laboratory-held pink shrimp, P. duorarum, in Texas (Johnson, 1974a), and from raceway-reared P. californiensis in Mexico (Lightner, in press). An additional Fusarium sp. had been described from the lobster, Homarus americanus, from a small experimental lobster farm in New England (Lightner, in press; Lightner and Fontaine, 1975). In all of the cases of Fusarium sp. infections of crustaceans, destruction or damage to the gills by the fungus has been reported.

The Fusarium sp. in P. japonicus caused a disease that was expressed as "black gills" and resulted in serious mortalities among pond-cultured prawn populations. Affected parts of the gills carried septate hyphae of the fungus. Intramuscular inoculation of healthy prawns with conidia of the fungus caused "black gill disease," and the fungus was isolated from gill lesions of artificially infected prawns (Egusa and Ueda, 1972).

Another Fusarium sp. that differs sufficiently from the Fusarium sp. of P. japonicus to represent a different species was responsible for a severe epizootic in raceway-reared California brown shrimp (P. californiensis) in June and July of 1974, at the experimental shrimp farm in Puerto Peñasco, Mexico. The disease was confined to two raceways and two small tanks at the farm, but nearly 100% incidence was observed. In one of the raceways, only 600 of the 6,000, 100-mm shrimp present in June survived to July 12. By late August, all but a few of the remaining 600 had died due to the disease. The disease was expressed grossly as blackened lesions in the gill region.

The fungus typically infected the gills, the coxal segments of the walking legs, and the body wall behind the gills and above the coxal segments. The coxal segments, gill processes, and adjacent

portions of the 14th body segment (the segment with the 5th walking legs) were nearly always infected by the fungus (Figure 1). In every instance, at least some of the fungal lesions on a particular shrimp were blackened by deposition of melanin. Hence, most of the affected shrimp showed at least a limited "black gill" condition. In these black lesions (Figure 2) the melanin deposition resulted from the activity of hemocytes responding to the presence of hyphae and to tissue destruction caused by the fungus. Encapsulation of hyphae was typical when hyphae were present in subcutaneous or muscle tissue. Death in affected shrimp, as with Fusarium infections in the Kuruma prawn and the lobster, probably resulted from destruction of the gills by a rapid antemortem growth of the fungus into the gill processes that was not accompanied by an appreciable hemocyte response (Figure 3).

The fungus was easily isolated and cultured from tissue samples taken from the gills of moribund shrimp that had lesions like those described. Isolation media was Sabouraud dextrose agar supplemented with 2% NaCl and with shrimp homogenate (SSS medium), and Cantino PYG broth supplemented with 2% NaCl. Penicillin and streptomycin were added to isolation media to inhibit bacterial growth. The Fusarium sp. from P. californiensis produced micro- and macroconidia in artificial media and in shrimp tissues. Microconidia were typically ovoid to oblong and frequently slightly curved (Figure 4); they were 1 or 2-celled, and ranged from 9 to 18 µm in length. Macroconidia were typically 3 or 4-celled and canoe shaped. Macroconidia ranged in length from 30 to 47 µm (Figure 5). The fungus produced a pale brown diffusable pigment on SSS medium. This pigment was much paler than the dark purplish-brown pigment produced by the Fusarium sp. from P. japonicus (Egusa and Ueda, 1972).

Control of this disease at Puerto Peñasco was accomplished by elimination of sources of spores of the fungus, destruction of shrimp infected with the fungus, and disinfection of affected tanks and raceways with 200 ppm chlorine (Lightner, in press).

Gill Disease Due to Epicommensal Protozoans

Johnson et al. (1973) reported the loss of an estimated 2,000 pond-held brown and white shrimp in a single day due to the presence of large numbers of Zoothamnium sp. (Figure 6) on the gills and due to a reduction in dissolved oxygen. Mortality was attributed to anoxia as the mortalities occurred when the infestation of the protozoan became heavy enough to restrict oxygen exchange and when the dissolved oxygen level in the ponds dropped to a low of 2.6 ppm. In ponds where no Zoothamnium sp. were observed on the shrimp, no mortalities occurred despite the low dissolved oxygen levels. Good survival has been experienced with P. aztecus in culture ponds even when the dissolved oxygen fell to 1 ppm (Johnson et al., 1973).

Histopathological lesions of the gills, appendages, or general body surface have not been demonstrated at the site of attachment of

a colony of Zoothamnium sp. (Overstreet, 1973). The stalks of colonies of this protozoan attach to the surface of the cuticle and do no mechanical damage to the cuticle. There is no foreign body response by the shrimp's hemocytes at the site of attachment (Figure 7). Death occurs when the effective respiratory surface of the gills is reduced by the presence of numerous colonies of Zoothamnium sp. and suffocation results. The process is passive and is probably aggravated by reduced dissolved oxygen concentrations in the water (Overstreet, 1973).

Successful control of Zoothamnium sp. on penaeid shrimp in ponds with formalin at 25 ppm was reported by Johnson et al. (1973). Other experimental treatments that used a lower concentration of formalin (15 ppm), potassium permanganate at 2 and 4 ppm, copper sulfate at 1 ppm, or malachite green at 1 ppm were not effective in killing or removing Zoothamnium colonies from the shrimp's gills.

A loricate peritrich, probably a Lagenophrys sp., has been observed on the general body surface of pond-reared shrimp (P. setiferus and P. vannamei) in Texas (Johnson, 1974b). At Galveston a similar Lagenophrys sp. was observed on the gills of white and brown shrimp. When present on the gills, Lagenophrys sp. differs from Zoothamnium sp. by evoking a strong cellular inflammatory response. Individual trophonts of Lagenophrys sp. typically attach near the tips of the gill lamellae (Figure 8). While no portion of the lorica appears to penetrate or damage either the cuticle or the underlying hypodermis of the lamella the site of attachment becomes heavily inflamed and congested with hemocytes (Figure 9). Often the hemocyte accumulations become melanized. A similar process of inflammation by hemocytes was noted in the processes of wound repair and foreign body elimination in the white shrimp (Fontaine and Lightner, 1973, 1974). Shrimp having heavy infestations of Lagenophrys sp. on the gills display a "black gill" condition. In such animals, numerous gill lamellae and often large portions of a whole gill process are heavily congested with hemocytes, are melanized, and are nonfunctional. Hence, the respiratory capacity is reduced, and in severely affected animals, death due to suffocation may result if tissue oxygen demands increase (e.g., following handling stress or immediately prior to molting) or if dissolved oxygen levels decrease.

Filamentous Gill Disease

Leucothrix mucor and Leucothrix-like filamentous bacteria have been reported from numerous crustaceans. The presence of Leucothrix mucor has been demonstrated on the eggs of the rock crab (Cancer irroratus), on the setae of the pleopods of the grass shrimp (Palaemonetes pugio), and on the pleopods of the green crab (Carcinus maenas) (Johnson et al., 1971). Leucothrix-like filamentous bacterium have also been reported on the general body surface and on the gills of three penaeid species (P. vannamei, P. stylirostris, and P. setiferus) from rearing ponds in Texas (Johnson, 1974b).

Barkate et al. (1974) reported mortalities in postlarval penaeid

shrimp due to a large filamentous bacterium. During early stages of development, postlarval shrimp became entangled in filaments of the bacterium, resulting in stress to the shrimp. Heavy mortalities (30% to 100%) were experienced, usually suddenly and without warning except for a foul sewage-like odor in the tank. This filamentous bacterium apparently grew on waste materials on the bottom of tanks and was visible as white, cottony mats on the surface of the sediment. The organism was successfully isolated and cultured, but failed to produce filaments on culture media. Reversion to the filamentous state was reportedly not accomplished unless the cultures were inoculated back into the shrimp environment (Barkate et al., 1974). In culture Barkate's isolate produced spore-forming rods that stained Gram-positive to Gram-variable. The isolate was tentatively identified as Bacillus cereus var. mycoides.

A similar filamentous organism was observed on the gills of juvenile penaeid shrimp in rearing tanks in Florida, and this organism was reportedly isolated on solid media. However, attempts to infect healthy shrimp using "infested tank sediment" rather than the isolate from the gills were not successful (Barkate et al., 1974).

Sporadic but serious epizootics due to a Leucothrix-like filamentous organism have occurred in tank and raceway-reared P. californiensis at the experimental shrimp farm in Puerto Peñasco, Mexico. Periodic sampling of affected populations of shrimp from the tanks and raceways at Peñasco revealed that the filamentous organism was typically present on the pleopods and gills. When the filamentous organism became so abundant on the gills that respiration was blocked, mortality occurred (Figure 10), despite apparently adequate dissolved oxygen levels.

The filamentous organism at the Puerto Peñasco shrimp farm was initially thought to be either a species of the Oscillatoriaceae group of the blue-green algae like that which reportedly occurs on Crangon crangon (Shelton, 1974) or a filamentous bacterium. The latter is believed to be the case because of the close morphological similarities of this organism to Thiothrix marina (Harold and Stanier, 1955), to Leucothrix mucor (Johnson et al., 1971; Bland and Brock, 1973), and to Barkate's isolate (Barkate et al., 1974).

Histological studies performed on California brown shrimp (P. californiensis) from the Puerto Peñasco facility and on brown and white shrimp from Galveston, Texas, revealed that the filamentous organism was strictly external. The organism was attached to the cuticular covering of the gill lamellae, pleopods, or other appendages, and its presence did not result in demonstrable histological damage to underlying tissues (Figure 11). The filaments themselves appeared to be segmented and had the general appearance of a string of tubular beds (Figure 12), particularly in histological preparations. The filament segments were anucleate. Trapped in the "mat" formed by the filaments were abundant amounts of detritus, some filamentous green algae, and often numerous diatoms. By itself the

filamentous organism is not responsible for discoloration of the gills, but the accumulation of algae and debris trapped by the filaments results in a gross discoloration of the gills. This discoloration ranges from pale brown to black or green if sufficient algae is trapped by the filaments.

The conditions responsible for the presence of the filamentous organism on the shrimp's gills have not been determined, although low dissolved oxygen levels seem to favor development of the disease, as well as contributing to mortality once the disease has become established. Mortality of shrimp having heavy infestations of the filamentous organism on the gills usually occurs during or immediately following molting. Many premolt animals that showed heavy gill infestations did not survive the next molt if left untreated, despite apparently adequate dissolved oxygen levels in the water. Animals that did survive the molt shed the filaments with the cast exoskeleton and remained free of filaments for at least a few days.

Attempts to culture the organism causing filamentous gill disease have been made using numerous media, some that were intended for culture of blue-green algae, some for fungi, and the remainder for various types of bacteria. Unfortunately, no organism has been cultured that produces filaments like those seen in filamentous gill disease when grown on artificial media.

Shrimp having filamentous gill disease have been treated successfully with 5 to 10 ppm potassium permanganate in 1-hour static treatments. The use of 10 ppm potassium permanganate has occasionally resulted in a chemical toxicity expressed as "burned" gill lamellae. Such lamellae were initially brown but later became melanized and necrotic. Croghan (1958) noted a similar phenomenon in brine shrimp (Artemia salina) following a 5-min exposure in a saturated potassium permanganate solution. The survivors of such treatment displayed a browning ("burnt" appearance) and distortion of the epithelium of the first 10 pairs of branchiae. In these survivors the damaged branchial epithelium underwent a further slow degeneration and blackening, probably due to deposition of melanin (Croghan, 1958).

A concentration of 5 ppm potassium permanganate removed or greatly reduced the bacterial filaments on the gills in P. californiensis, but unfortunately within 5 to 10 days filaments reappeared and mortalities began again. Other chemical treatments including a mixture of malachite green oxalate and formalin at 0.05 to 0.1 ppm and 25 to 75 ppm, respectively, and Hyamine 3500 at 1 to 2 ppm have not been beneficial in 1-hour static treatments. Chemicals such as methylene blue, Roccal, copper sulfate, and Furanace are presently being tested as are 24-hour flow-through treatments using 1 to 2 ppm potassium permanganate.

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